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Chorionic Villus Sampling and Transverse Digital Deficiencies: Evidence for Anatomic and Gestational-Age Specificity of the Digital Deficiencies in Two Studies

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Several but not all studies indicate that chorionic villus sampling (CVS) is associated with an increased risk for transverse limb deficiencies, including digital deficiencies. It has been suggested that variations in results regarding the transverse digital deficiencies (TDDs) may be due to the use of different classification criteria. We present the combined analysis of two case-control studies, the U.S. Multistate CVS (US) study and the Italian Multicentric Birth Defects (IPIMC) study, using two different definitions of TDDs. We compared the frequency of CVS exposure in control infants with that among those infants with any number of affected digits (any TDD), and those with all five digits of at least one limb affected (extensive TDDs). The estimated relative risk (RR) for any TDD following CVS was 10.6 (IPIMC) and 6.6 (US). For the extensive TDDs, the RR was 30.5 (IPIMC) and 10.7 (US). In both studies, extensive TDDs were less than 25% of all TDDs. Compared to all TDDs, extensive TDDs were more likely to occur after CVS performed earlier in the first trimester (before 10–11 weeks' gestation). These findings suggest a relationship between the timing of CVS and the severity of TDDs; indicate that using a restrictive definition of TDDs (all five digits affected) may limit the ability to evaluate the association between CVS and TDDs in populations in whom CVS is usually performed at or after 10 weeks' gestation; and highlight the necessity to consider gestational age in any evaluation of the relative risk for limb deficiencies associated with CVS.

KEY WORDS: limb deficiency, malformation, birth defect, prenatal diagnosis, chorionic villus sampling, case-control study, epidemiology

INTRODUCTION

Chorionic villus sampling (CVS) has been shown to be associated with an increased risk for transverse limb deficiencies in several studies [Firth et al., 1991; Burton et al., 1992; Brambati et al., 1992; Mastroiacovo et al., 1992, 1994; GIDEF, 1994; Olney et al., 1995], but not in all [Jackson et al., 1993; Froster and Jackson, 1994]. In those studies in which an association was found, it was consistently specific for transverse limb deficiencies, as reflected by the high relative risk for these but not for other types of limb deficiencies. The level of the lesions included essentially the whole spectrum of severity, from severe proximal lesions to milder digital deficiencies. The extent and level of limb involvement depend, at least in part, on the timing of the exposure. It has been shown that digital deficiencies were more common than the more severe limb anomalies among subjects exposed to CVS at a later gestational age [Firth et al.,

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1994]. However, there is some concern that a selection bias or variations in classification criteria may account for some of the apparently contradictory results among some of the studies, in particular those regarding digital deficiencies. For instance, some argue that transverse digital deficiencies should be defined as deficiencies involving all five digits of a limb [Forster-Iskenius and Baird, 1989], while others have used less restrictive criteria and include cases involving fewer than five digits [Temtamy and McKusick, 1978; Källén et al., 1984]. The strength of the association with CVS could depend on which criteria are used in defining the case group. To our knowledge, the association of CVS with specific subgroups of transverse digital deficiencies has not been studied by other investigators, though it may have important methodologic and pathogenetic implications.

To explore this issue, we present the combined analysis of two studies, the U.S. Multistate CVS (US) Study [Olney et al., 1995] and Italian Multicentric Birth Defects (IPIMC) Study [Mastroiacovo and Botto, 1994]. Both of these case-control studies previously identified a strong association between CVS and transverse limb deficiencies. However, only the US study examined the transverse digital deficiencies separately, and neither investigated the association of CVS with different subgroups based on the extent of the digital involvement. In this combined analysis, we wanted to evaluate in both populations whether CVS was a risk factor for digital deficiencies, regardless of whether all five digits were involved. We also wanted to investigate whether the extent of digital involvement was related to the timing of the procedure.

METHODS

We used data from the US and the IPIMC studies. In the US study, case subjects were infants with a nonsyndromic limb deficiency, ascertained by population-based birth defect surveillance and born from 1988 through 1992 to mothers 34 years old or older at the time of conception. Control subjects were infants with other major birth defects and without limb deficiencies, matched to case subjects by infant's year of birth and mother's age, race, and state of residence. Case and control subjects were ascertained from existing files of seven birth defect surveillance programs, including those in Metropolitan Atlanta and the states of Arizona, California, Illinois, Maryland, New Jersey, and New York. These programs have independently collected birth defect data for the past 7 to 27 years. Case ascertainment within each state is either "active" (in which program personnel systematically seek and abstract medical records in the field) or "passive" (in which health care providers report defects). Case and control infants were live- or stillborn, with gestational ages of at least 20 weeks or birth weights of at least 500 g. Additional information on the methods of this study has been published [Olney et al., 1995]. In the IPIMC study, case subjects were infants with a nonsyndromic limb deficiency, ascertained by a hospital-based birth defect surveillance registry and born from 1988 through 1994. Control subjects were infants with any other major birth defect. The IPIMC registry has been in operation since 1978 and ascertains infants with birth defects during the neonatal period. Case and control infants were live- or stillborn with gestational ages of at least 20 weeks or birth weights of at least 500 g. Additional information on the IPIMC methods has been published [Mastroiacovo et al., 1994].

For all case subjects from both studies, we systematically reviewed the description of limb defects from a physical examination and from reports by surgeons, radiologists, geneticists, or pathologists. From the original case groups, we selected all infants with a transverse digital deficiency, which we defined as the absence of all or part of any phalanx with intact metacarpal or metatarsal bones and proximal segments. We excluded cases involving infants with isolated deficiencies of the first digit (thumb or toe). The remaining infants constituted the case group in our analyses. We then classified the transverse digital deficiencies either as deficiencies affecting up to four digits or as deficiencies affecting all five digits, and divided the latter group into "certain" and "probable" cases, depending on whether the description in the medical record was conclusive or only highly suggestive for the diagnosis. As a measure of association, we used unmatched crude odds ratios (ORs) and 95% exact confidence intervals (CIs), which we computed using the Fisher exact algorithm module in the software package SABER (written by Levy James, Centers for Disease Control and Prevention, Atlanta, GA). The attributable fraction among cases (AFc), that is, the proportion of affected infants exposed to CVS in whom the exposure caused the outcome, given that the association between exposure and outcome is causal, was derived from the OR as follows: $AFc = (OR - 1)/OR$.

RESULTS

Table I shows the distribution of case and control subjects whose mothers had been exposed to CVS. The proportion of control mothers exposed to CVS was 0.47% (44/9,430) in the IPIMC study, and 5.88% (6/102) (among mothers 34 years of age and older at conception) in the US-Multistate study. In both studies, CVS was associated with a markedly increased risk for transverse digital deficiencies, regardless of whether all five digits were involved or not. Selected characteristics of the infants with transverse digital deficiencies whose mothers had undergone CVS are listed in Table II. Using the less restrictive definition (any number of digits involved), we found that the estimated relative risk for CVS was 10.6 in the IPIMC study and 6.6 in the US study (Table I). Using the more restrictive definition (all five digits of at least a limb involved), we found that the estimated relative risk for CVS was 30.5 in the IPIMC study and 10.7 in the US study. For transverse digital deficiencies affecting fewer than five digits, the estimated relative risk was 8.8 in the IPIMC study and 6.2 in the US study. These findings did not change appreciably when probable cases were included in the case group. When we plotted the cases of transverse digital deficiencies exposed to CVS by the gestational age at which the infants had been exposed to the proce-

TABLE I. Estimated Relative Risk for Transverse Digital Deficiencies (TDDs) Following Chorionic Villus Sampling, by Extent of Lesion (IPIMC, 1988–1994, and US-Multistate, 1988–1992)

	Italy-IPIMC				US-Multistate			
	CVS	No CVS	OR	95% CI	CVS	No CVS	OR	95% CI
Controls	44	9,386	Ref	—	6	96	Ref	—
Cases								
All TDDs	6	121	10.6	3.6–25.5	7	17	6.6	1.6–26.4
TDDs all five digits								
Definite[a]	2	14	30.5	3.3–138.6	2	3	10.7	0.7–100.6
Definite + Probable[a]	2	24	17.8	2.0–75.2	2	4	8.0	0.6–67.7
TDDs 1 to 4 digits	4	97	8.8	2.3–24.9	5	13	6.2	1.3–27.7

[a]Definite: description explicitly defined the extent and type of lesion; probable: description highly suggestive but not definite for the diagnosis of TDD affecting all five digits. CVS, exposed to CVS; no CVS, not exposed to CVS; OR, odds ratio; 95% CI, 95% confidence interval.

TABLE II. Transverse Digital Deficiencies Among Subjects Exposed to Chorionic Villus Sampling, Italy-IPIMC, 1988–1994, and US-Multistate, 1988–1992

Digital deficiencies	Other defects	Gestational age[a]	Study[b]	Classification[c]
All toes absent or hypoplastic on one foot	—	8 weeks	US	TDD-5
Absent distal phalanx of thumb, absent second finger, absent middle and distal phalanges of 3rd, 4th, and 5th finger of one hand	Micrognathia, asymmetric facies, paralysis of 6th and 7th cranial nerves	8 weeks, 1 day	IPIMC	TDD-5
Hypoplastic distal phalanges of all fingers except thumb, both hands	—	9 weeks, 2 days	US	TDD-4
Absent distal phalanges of 2nd, 3rd, 4th, and 5th toe of one foot	Micrognathia, microglossia, dysmorphic facies	9 weeks, 4 days	IPIMC	TDD-4
Absent distal phalanx of 4th finger, absent nail on 3rd finger	—	10 weeks, 0 days	US	TDD-4
Absent distal phalanx of middle finger of one hand	—	10 weeks, 0 days	US	TDD-4
Absent distal phalanx of 5th finger of one hand	—	10 weeks, 3 days	IPIMC	TDD-4
Absent distal phalanx of 2nd finger, distal and middle phalanges of 3rd and 4th finger of one hand, with hypoplasia of hand and arm	—	10 weeks, 4 days	IPIMC	TDD-4
Absent phalanges of 2nd, 3rd, 4th, and 5th finger of one hand, absent distal phalanges of 1st, 2nd, and 4th toe and distal and middle phalanges of 3rd toe of one foot, absent distal phalanges of 2nd and 3rd toe of the other foot	—	10 weeks, 4 days	IPIMC	TDD-4
Absent distal phalanges of all toes of one foot	—	10 weeks, 5 days	IPIMC	TDD-5
No toes on either foot, absent (unspecified) fingers on both hands	—	10 weeks, 6 days	US	TDD-5
Absent distal phalanges of one hand	Micrognathia, low-set ear	10 weeks	US	TDD-4
Absent distal phalanges on toes of one foot	Sacral dimple, nevus on neck	11 weeks	US	TDD-4

[a]Gestational age, time elapsed since last menstrual period. Based on the date of the procedure as reported on medical records, on mother's report, or estimated by sonographic parameters on the date of the procedure.
[b]US, US-Multistate study; IPIMC, Italy-IPIMC study.
[c]TDD-5, *all digits of at least one limb affected*; TDD-4, no more than four affected digits on any limb.

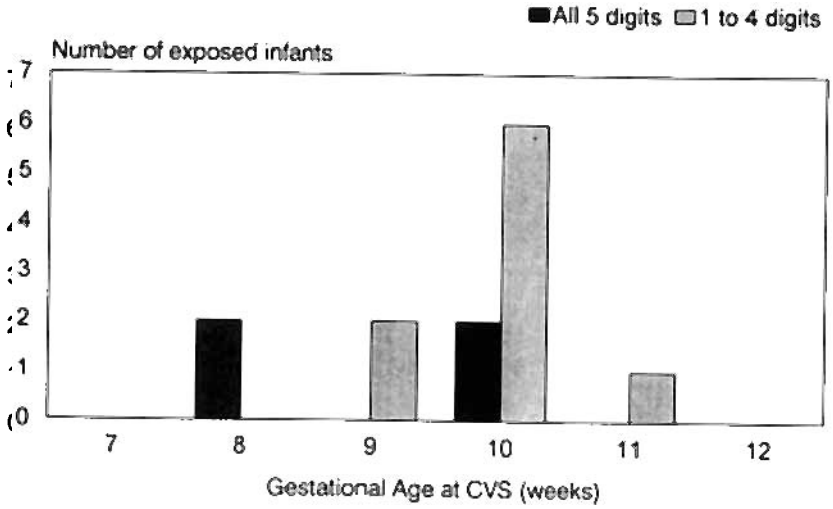

Fig. 1. Infants with transverse digital deficiencies exposed to CVS, by timing of procedure and extent of digital involvement (Italy-IPIMC, 1988–1994, and US-Multistate, 1988–1992).

dure (Fig. 1), we found that subjects with all five digits affected tended to have been exposed to CVS at an earlier gestational age than those with fewer affected digits. All subjects (2/2) with a transverse digital deficiency who had been exposed to CVS before 9 weeks of gestational age had 5 digits affected. This proportion decreased to 50% (2/4) among those exposed before 10 weeks and to 33% (4/12) among those exposed before 11 weeks. No case of such a severe transverse digital deficiency occurred among subjects exposed to CVS at 11 weeks of gestational age or later.

DISCUSSION

Our findings confirm in a second population (Italy-IPIMC) that exposure to CVS is associated with an increased risk for transverse digital deficiencies. Furthermore, in both studies the estimated relative risk is elevated regardless of whether the definition of transverse digital deficiencies includes only those cases in which all five digits are involved or whether the definition is expanded to include also those cases involving fewer than five digits. However, the relative risk is higher in the subgroup with all five digits affected. The severity of the digital deficiencies may be related to the timing of CVS during gestation: in our combined series the subjects with all five digits affected tended to have been exposed to CVS at an earlier gestational age than those with fewer involved digits.

In evaluating these results, we must assess the potential limitations of our study. Biased ascertainment of case and control subjects is always a concern in retrospective studies. In this setting, for instance, one could argue that the publicity around the suggested relationship between CVS and transverse limb deficiencies may have introduced a selection bias by increasing selectively the ascertainment of infants with transverse digital deficiencies who had been exposed to CVS. However, most of the study population was ascertained before the association between CVS and limb defects was first suggested [Firth et al., 1991], so we think that selection bias is not a likely explanation for our findings. Moreover, the selective reporting would likely have a greater effect on reports of cases of the less severe defects, defects that perhaps would have not been reported had the affected infants' exposure to CVS not been known, and would thus result in a higher risk associated with the milder deficiencies than with those affecting all five digits. There is no evidence of this in our findings, where in fact the risk associated with CVS is higher for transverse digital deficiencies affecting all five digits than for those involving fewer than five digits. Another potential limitation of our study is the relatively small sample size. Transverse digital deficiencies are relatively rare, and the sparsity of the data, particularly in the subgroup analysis, is reflected by the wide confidence intervals around the estimated relative risks. Among the strengths of our combined analysis is the case-control design of the two studies. The case-control approach was advantageous, because the outcome is rare. In fact, these two studies were based on surveillance systems that monitored a combined population of approximately one million births [Olney et al., 1995; Mastroiacovo et al., 1994]. The study mothers in the US-multistate study were restricted to those 34 years of age or older, whereas no such restriction was used in the Italy-IPIMC study. This difference in study population is reflected in the prevalence of CVS use among the control mothers, that is much higher in the US-multistate study than in the Italy-IPIMC study (5.88 vs. 0.47). Despite the differences in the populations under study and in the methodologies used, the results of the two studies are strikingly concordant, thus adding to the plausibility and generalizability of our findings.

Our findings have several methodologic and pathogenetic implications. First, if the association with CVS is causal, the high relative risk for transverse digital deficiencies (10.6 in the IPIMC, 6.6 in the US) implies that in most affected infants exposed to CVS, the exposure caused the defects and therefore most of these defects could have been prevented had the exposure not occurred [Meittinen, 1974]. In fact, the attributable

fraction among the exposed cases, that is, the proportion of infants with any transverse digital deficiency exposed to CVS in whom the exposure caused the digital deficiencies given that the association with CVS is causal, was 91% in the IPIMC study and 85% in the US study. The attributable fraction among cases increases with increasing OR, and therefore the increased risk associated with the infants with all five affected digits is likely to reflect a marked specificity of the lesions induced by CVS. Moreover, if the mechanism of action of CVS involves a vascular disruption, as has been suggested for some transverse limb deficiencies [Hoyme et al., 1982; Brent, 1993; Lipson and Webster, 1993], then our findings suggest that vascular disruptions cause a higher proportion of digital deficiencies involving all five digits than those involving fewer digits.

Second, the findings that the extent of digital involvement is related to the timing of the procedure extends to subgroups of transverse digital deficiencies the relationship between the severity of defects and the timing of the procedure, already noted by several authors for the more severe limb defects and summarized by Firth et al. [1994]. Thus, our findings add to the biologic plausibility of the association between CVS and these limb deficiencies.

Third, these findings suggest that careful attention should be given to the tension between specificity of the outcome and the timing of CVS in the design and analysis of such studies. On the one hand, it has been shown that generally in the study of birth defects, the effect of an association is more clearly seen when subgroups with increasing pathogenetic homogeneity are used for analysis [Friedman, 1992; Khoury et al., 1992]. For example, thalidomide causes a variety of limb defects but it is most strongly associated with intercalary defects; similarly, diabetes causes many types of congenital heart defects, but it is more strongly associated with conotruncal heart defects; moreover, CVS is associated with an increased risk for transverse limb deficiencies but not for all limb deficiencies combined [Olney et al., 1995], and this finding could explain the lack of association between CVS and limb anomalies in those studies that did not evaluate the risk for transverse limb deficiencies but only the overall risk for all limb deficiencies combined [Dolk et al., 1992]. In the same way, the high relative risk for transverse digital deficiencies affecting all five digits found in our two studies would seem to suggest that this outcome, while present in less than 25% of all infants with digital deficiencies in these two studies, would be the most specific one associated with CVS, and therefore indicate the need for a restrictive definition. On the other hand, the finding that these defects are seen mainly among infants exposed to CVS at an earlier gestational age indicates that the association of CVS with specific outcomes cannot be evaluated accurately without considering gestational-age distribution at which the population under study is exposed. For example, in a population in which most women undergo CVS at or after 10 weeks of gestation (currently the most common time of the procedure), using the restricted definition of transverse digital deficiency (all five digits affected) will focus on only a small proportion of digital deficiencies associated with that particular timing of the exposure, thus increasing the likelihood of missing a true adverse effect. In addition, the degree of distortion of the estimated relative risk may depend also on the design of the study. The case-control approach is probably less likely to mask an effect even when a restrictive definition is used: since this study design is particularly efficient for rare outcomes, the increased specificity of the definition often offsets the decrease in the number of cases needed to find an effect. On the other hand, follow-up (cohort) studies require substantially more study subjects to have sufficient statistical power to detect associations between causes and rare outcomes, and restricting the definition of transverse digital deficiency to those involving all five digits is likely to decrease the number of outcomes under consideration, and increase the number of individuals needed to suggest or exclude a certain effect of CVS.

In summary, our findings indicate that CVS is associated with an increased risk for transverse digital deficiencies, using two different criteria for classification. The estimated risk is particularly high for lesions involving all five digits of a limb, although they were a minority of all cases of TDDs. We also found that infants with all five digits affected tended to have been exposed to CVS at an earlier gestational age compared to those with fewer digits affected. These findings suggest a relationship between the timing of CVS and the severity of TDDs; indicate that using a restrictive definition of TDDs (all five digits affected) may limit the ability to evaluate the association between CVS and TDDs in populations in which CVS is usually performed at or after 10 weeks' gestation; and highlight the necessity to consider gestational age in any evaluation of the risk for limb deficiencies associated with CVS.

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